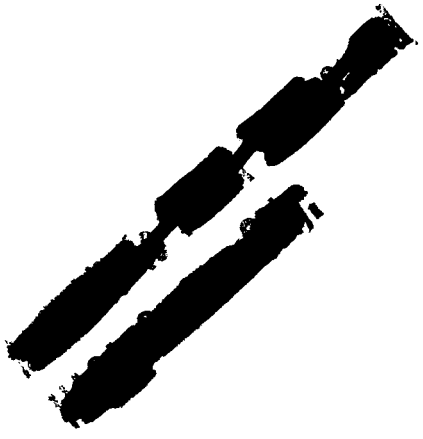
NSG-689

Experiments Suggesting Evolution to Protein[1]



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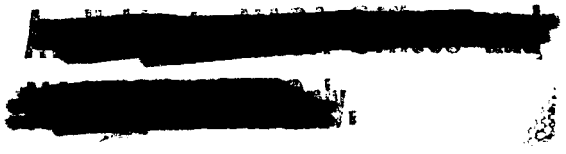
J. D. Bernal has stated recently, "Questions of origin have a logic of their own" (1). The special logic of questions of origin applies to, and was applied by Bernal to, methods of answering those questions. When those questions are answered, one should anticipate that the answers must not have an existence of their own, but should ultimately fit into a conceptual continuum. When the questions concern the origin of protein, or of other biochemical systems, the continuum must span prebiological molecular evolution and Darwinian organismic evolution unless one assume a discontinuity between pre-life and life. Theoretical constructions which do not comport with pertinent parts of such a continuum can hardly be valid models. For instance, the validity of any simulated primitive synthesis of amino acids should be evaluated by the ease with which the process can be reconciled with the necessary subsequent step of condensation polymerization.



Experiments which have been performed in the context of the origin of life, or of the origin of protein, have several relationships to the subject matter of this conference. These relationships involve a) the background of knowledge of evolution of protein molecules in organisms, which provides clues to the conceptual origin of protein[2/] (7), b) the employment of knowledge of the properties of contemporary protein as a test of the validity of experimental models of primitive protein, and c) conceptual answers to some of the problems of primordial protein. The first protein was of course the starting point for evolving proteins. The original protein may well have been closely related to the starting point of gene-controlled enzymes and of polymerases. One purpose of this paper will be to suggest the relationship of earliest protein to the protocell. Both proteins and genes may have required the simultaneous evolution of the cell (cf. 28).

Studies in our laboratory of the organismic evolution of proteins began with considerations of applying sequential and terminal residue methods to tracing evolution of primary structure of protein molecules (5,7,15,43). Porter and Sanger were the first to provide information for such possibilities (39). Some of the concepts which emerged from these studies in our laboratory were: a Darwinian explanation of micro-heterogeneity (6) of protein preparations (again recently shown

not to be entirely explainable as artifacts (4), demonstration that the evolution of protein has proceeded to yield only a minute fraction of the theoretical possibilities (7), an explanation of the similarities between protein molecules (7,15) as related to slow stepwise substitution of residues, the use of such techniques in chemical taxonomy (5), detailed analyses of genealogy of homologous and heterologous proteins (43), etc.

Studies of genealogical relationships of proteins posed the possibility that the results might harbor clues of the origin of the first protein. In particular, the presence of disproportionately large ratios of the dicarboxylic amino acids (as acids plus amides) invited attention. This feature suggested the possibility that these contents are an evolutionary reflection of relatively high proportions of these amino acids in the first protein and correspondingly, of the molecular matrix which may have yielded the first protein (7). Experiments were accordingly constructed to test the effect of sufficient proportions of glutamic acid, glutamine, aspartic acid, and asparagine (17) on the anhydrocopolymerization of amino acids. When sufficient proportions of these were heated in dry mixtures of the eighteen amino acids common to protein, genuine polyanhydro-α-amino acids resulted (17,18). This result is in contrast to the long known pyrolytic behavior of amino acids as depicted on the left in Fig. 1. Clean polymers

can be produced by heating dry in the molten state to temperatures such as 170° C followed by purification of the products from water through salting out with ammonium sulfate (right side of Fig. 1). The polymer shown on the right in Fig. 1 can be produced from mixtures of the eighteen amino acids common to protein; the polymers contain some of each of the eighteen, plus amide groups. The polymerization under these conditions can accomodate more or less than eighteen types of amino acid. In such heteropolyamino acids, the proportion of each neutral or basic amino acid can be high enough that it overlaps the proportion found typically in proteins (24).

Although the proportion of aspartic acid tends to be considerably above the usual percent found in protein, fractions with less than 20% aspartic acid have been isolated (36). For those polymers having relatively large proportions of aspartic acid, the polymer is of course acidic in reaction. These acid protein-like polymers (acid proteinoids) typically have weights of many thousands. Their properties have been studied extensively and reported a number of times. In this paper, the key properties which have been compared with those of protein are collected, with relevant references, in Table I.

In comparing the thermal proteinoids with contemporary proteins, one should recall that, inasmuch as no one protein has all of the pertinent structure and properties usually imputed to proteins as a class, a comparison with proteinoids

Table I

Properties of Thermal Poly-α-Amino Acids Compared to Properties of Proteins

Property	Reference
Qualitative composition	17,18,24,33
Quantitative composition	18,19,29,30,33
Range of molecular weight	18,22
Color tests	17,18,33
Solubilities	17,18,33
Inclusion of nonamino acid groups	22
Optical activity	22,40
Salting-in and salting-out properties	18,22
Precipitability by protein reagents	18,33
Hypochromicity	40
Infrared absorption maxima	18,22
Recoverability of amino acids on hydrolysis	24
Susceptibility to proteolytic enzymes	18,33,34
Catalytic activity	22,40
Inactivatability by heating in aqueous solution	40
"Nonrandom" (nonuniform) sequential distribution of residues	9,10,23
Nutritive quality	18,35
Morphogenicity	10

requires that the latter be scrutinized also as a class. In the case of each class of polymer, more data are clearly yet to be accumulated.

Besides comparing proteinoids with contemporary protein, one may attempt also to compare proteinoids with primitive organismic protein. Since no sure example of primitive protein exists, this comparison requires an indirect approach. The approach can conceptually be made through theoretical identification of the evolutionary pathways leading to and from primordial protein. Difficult as this may be to do with certainty, interpretable data are at hand. Of the many laboratory models of the prebiological synthesis of amino acids, one has been found to produce almost all of the amino acids common to protein and no other amino acids. This model is the thermal one, which, from a geological perspective, comports most easily with a continuum in which the next evolutionary step of anhydrocopolymerization is also thermal (31).

Properties of protein that one would ordinarily think of, and which have not yet been shown to be present in the thermal poly-α-amino acids, are antigenicity and helicity. Hypochromicity has been found, however. This hypochromicity has been shown to be correlated with the splitting in water of somewhat unstable aspartimide linkage (40), so the question of helicity is yet unanswered. The properties of antigenicity and helicity have been shown to be controllably introduced into

the Leuchs type of poly-α-amino acid (41); attempts to find these in thermal polymers have been incomplete as yet. Some proteins lack these properties. Accordingly, attempts to distinguish biosynthetic protein on Mars from spontaneously generated thermal poly-α-amino acids (27) by remote monitoring of tests could not be based on any qualitatively distinctive criteria. The similarities of the chemically synthetic and the biocynthetic polymers are emphasized by this evaluation in a context of comparative planetology.

Recent data which are not documented to the degree of those in the table is the finding that thermal proteinoids have weak but appreciable activity for the breakdown of a natural substrate, D-glucose, to glucuronic acid and that this product is then decarboxylated (25). These weak activities were reliably demonstrated with aseptically prepared proteinoid and U-C-14 glucose. Weak catalytic activities sufficient to launch an evolutionary succession of organisms is all that would be needed at first. The evolutionist would anticipate that such primitive activities would be enriched by Darwinian selection to provide the powerful enzymes of contemporary organisms (3).

Another unpublished study reported to the International Congress of Biochemistry in 1964 (36) included the report that a crude proteinoid can be fractionated on columns to yield many fractions each of which contain all of the amino acids common both to protein and to the unfractionated proteinoid.

The geological locales in which the anhydropolymerization of "spontaneous" amino acids could occur have been discussed (8,12). The ways in which the amino acids themselves might arise geologically have been reviewed (11). A salient feature of the experiments suggesting the origin of amino acids is the frequency and extent to which the key aspartic acid appears among the reaction products (27).

The property listed last in Table I is that of morphogenicity. The self-organizing properties of macromolecules have been invoked in a biological context (28). Wald has in particular suggested that these may have been crucial in the emergence of the first cell (44). Also remarkable was the foresight of C. R. Darwin who stated in 1871,

> "It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity & C present, that a proteine compound was chemically formed ready to undergo still more complex changes . . . " (10)

Darwin's remarkable insight is borne out by the model if one defer the pond until the "proteine compound" is formed in an anhydrous or hypohydrous environment. By intrusion of water into the hot mixture of anhydropolymerized amino acids, vast numbers of microscopic units having many of the properties of cells are formed (14). These properties cannot be adequately

evaluated without review of the visible consequences of these and other simple experiments. Such pictures are now in the literature. Two examples will be presented here. The quite fully documented properties of these units are set forth in Table II. A study of these properties indicates that natural experiments could have converted "primordial gases" such as methane, ammonia, and water to amino acids, amino acids to early protein, and the primitive protein to protocells with which natural experiments could have continued (28).

Two examples of the kind of self-organizing propensity in the thermal poly-α-amino acids are presented. In Fig. 2 is seen a photomicrograph of proteinoid microspheres in a suspension in which the pH of 3.0 has been raised by allowing a drop of McIlwain buffer of pH 6.5 to diffuse in under the cover glass of a microscope slide. The self-organized microspheres (27) are thus reorganized (28). The relationship depicted raises the question of whether the units appearing to have septa result from fusion or fission of the simpler spherical units. This question has been answered by many time-lapse cinematographic studies. The studies indicate that the process is fission (28) in these particular experiments.

Another example of self-organizing properties is found in Fig. 3. This photograph shows proteinoid microspheres which have undergone the pH increase of Fig. 2, and have been

Table II

Properties of Microparticles Spontaneously Organized From Thermal Poly-α-Amino Acids

Property	Reference
Stability (to standing, centrifugation, sectioning)	20,21
Microscopic size	10,20,21
Variability in shape Spheres, "buds", filaments	28
Uniformity of size	21
Numerousness	27
Stainability	26
Producibility as Gram-positive or Gram-negative particles	26
Solubility parallel to that of bacteria	26
Shrinkability in hypertonic solution	21
Swellability in hypotonic solution	21
Simulation of cell division	28
Electron micrographability	16
Presence of boundary	16
Selectivity of boundary	28
Bilamellarity of boundary	16
ATP-Splitting activity (by suitable inclusion of Zn)	13
Structured associations (algal-like)	27

stained with osmic acid, sectioned, and electron micrographed (16). In the photograph may be seen a double layer (16) such as had been believed to be unique to living cells (38). The examples presented illustrate the many cell-like properties found in and documented for the proteinoid microspheres. The experiments which serve as a model of evolutionary processes thus deal with evolution to and beyond the first protein.

Clearly, the proteinoid produced by heating differs from protein in at least the fact that the mechanism of its production is thermal. The synthesis of protein biologically requires, at least at present, ATP-dependent reactions. The way in which the more primitive kind of synthesis may have been supplanted by the more modern one is suggested by the fact that the known ATP-splitting ability of Zn salts (42) can be built into microspheres so that they split ATP at 40°. Such experiments have been performed in our laboratory by Joseph and Wiggert (13).

The fact that near-protein can be obtained in the fashion indicated resolves an old dilemma which has hindered the development of a theory of spontaneous generation. The concepts that a) the production of protein required the cell and that b) the production of cells required the preexistence of protein (2,32,37) has been a barrier to a conceptual continuum.

The pathway that has been suggested by laboratory studies involves first the production of molecules very much like those of protein, in the geological vessel rather than in a cell. The similarity of such poly-α-amino acid to protein is manifest in many characteristics, and especially in the tendency to form a kind of cell, at least as the cell is minimally defined. The experiments also demonstrate how such units can be made to approach, in steps, the viable contemporary cell. The basic dilemma of the evolutionary pathway to the first protein, and beyond to a precellular form, is now solved in principle.[3/] The sequence emerging from the experiments poses the possibility that the evolution of genes and enzymes should be examined in the context of evolving proteins, cells, and genes.

Acknowledgments

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Footnotes

1/ Work aided by Grants no. NsG-173-62 and NsG-689 of the National Aeronautics and Space Administration. Contribution no. 040 of the Institute of Molecular Evolution.

2/ Protein is used instead of the term proteins to connote the theoretical concept of a family of protein molecules related in an evolutionary manner.

3/ A thermal pathway to polynucleotides has been suggested also /Schwartz, A., and Fox, S. W., Biochim. et. Biophys. Acta, 87, 694 (1964)./

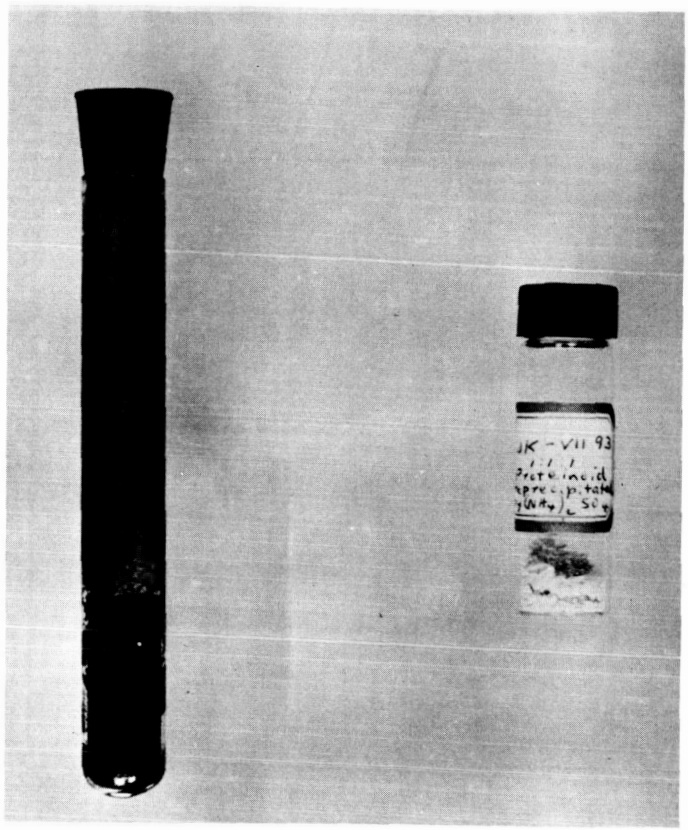

Fig. 1. The effects of heating dry amino acids above the boiling point of water. On the left - the usual result. On the right - with sufficient proportions of aspartic acid and of glutanic acid. This polyner has, in addition, been repurified by salting out the aqueous solution with ammonium sulfate.

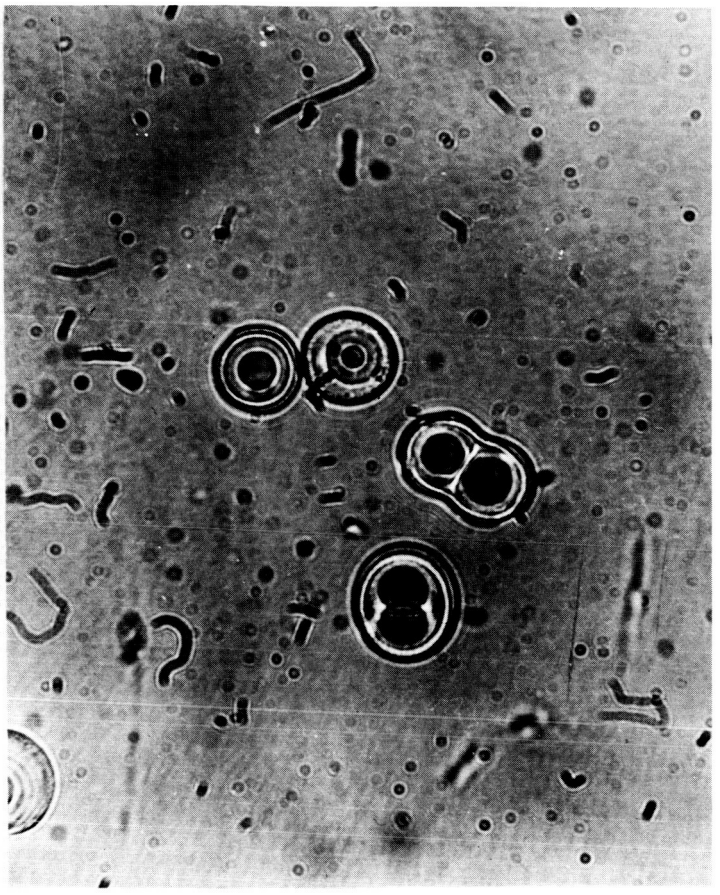

Fig. 2. Proteinoid microspheres in which septate division has been induced by elevation of pH.

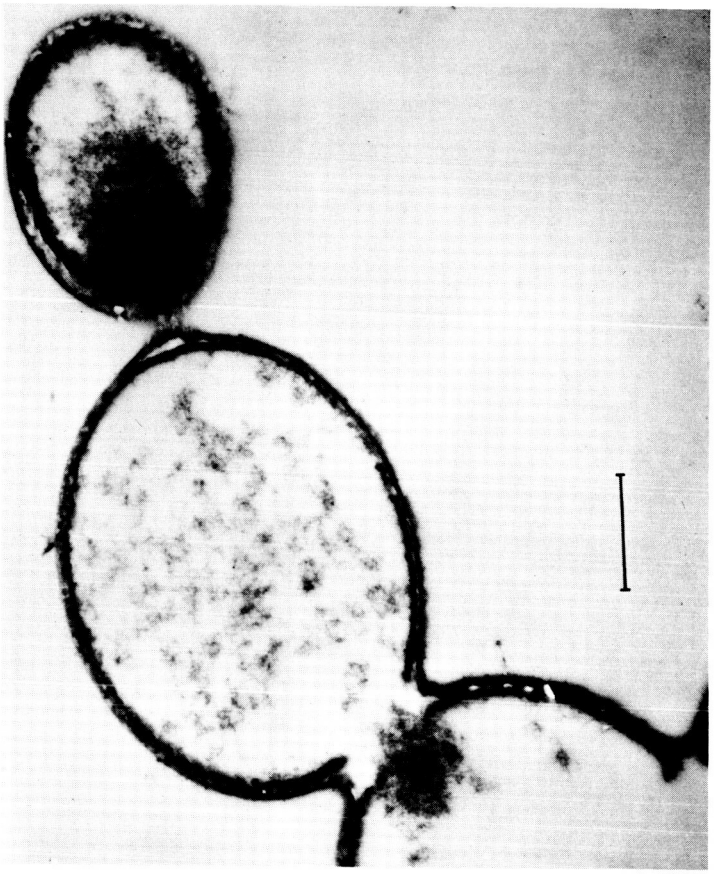

Fig. 3. Bilamellar boundary in electron micrographed section of osmic acid-stained thermal acid proteinoid microsphere. Marker indicates 1 micron.